# Euglenoids living in the intestines of microhylid tadpoles of Argentina

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**Large numbers of undamaged euglenoids (*Euglena ehrenbergii* var. *baculifera*, *E. spiroides*, *Lepocinclis fusiformis*, *L. salina*, *Phacus curvicauda*, *Trachelomonas bacillifera* var. *minima*, *T. pusilla* and *T. volvocina*) were found in the intestinal contents of tadpoles of *Dermatonotus muelleri* and *Elachistocleis bicolor*. Nine eggs of *Elachistocleis bicolor* were reared in the laboratory in plastic pools until the larvae reached stage 24. Seven of these tadpoles were placed in a glass container with water and a sample of phytoplankton rich in flagellated euglenoids (*Euglena ehrenbergii* var. *baculifera* and *E. intermedia* var. *klebsii*). Two control tadpoles were kept in the absence of euglenoids. Intestinal contents of the tadpoles were observed at stages 25, 27 and 36 (sensu GOSNER, 1960). *E. intermedia* var. *klebsii* was found in the intestinal tract of tadpoles of *E. bicolor*. All euglenoids were intact and had typical features of free-living cells in nature, with large accumulations of paramylon granules. We conclude that these euglenoids are not part of the diet of these tadpoles and that they are not digested.**

## INTRODUCTION

Protozoans, algae and nematodes have been reported or suggested as food items in diets of anuran tadpoles (HEYER, 1973, INGER, 1986). Most diet studies have been based on the taxonomic composition of items found in the intestinal contents of fixed specimens. We have found large numbers of euglenoids in the intestines of tadpoles of *Dermatonotus muelleri* and *Elachistocleis bicolor* collected in lentic environments from Argentina (unpublished data). These observations led us to study the euglenoid fauna in the larvae of *Elachistocleis bicolor* obtained from eggs developed in our laboratory and fed with freshwater euglenoids.

There are few reports on diet of microhylid tadpoles (LI & LIN, 1935; SAVAGE, 1952, HEYER, 1973; INGER, 1986; INGER et al., 1986, WANG et al., 1989). Almost all microhylid tadpoles lack keratinized mouthparts and are "filter feeding tadpoles, type 2" of ALTIG & JOHNSTON (1989). LI & LIN (1935) confirmed living euglenoids in the intestines of *Kaloula*

*borealis* and commented on the relationship between the protozoa and the tadpoles. Living euglenoids can survive passage through the intestines (HEGNER, 1926). LI & LIN (1935) noticed some digestion of euglenoids. *Euglenamorpha* and *Hegneria* are euglenoids that live in the hindgut of tadpoles (BRUMPT & LAVIER, 1924, WENRICH, 1924). The purpose of this paper is to determine whether tadpoles feed on euglenoids present in their intestines.

## MATERIALS AND METHODS

Two samples of tadpoles were examined and staged according to GOSNER (1960). The tadpoles have developed mouthparts and functional intestines in all stages used in this study.

Sample A Four tadpoles of *Dermatonotus muelleri* (stages 32, 33, 34 and 36) and two tadpoles of *Elachistocleis bicolor* (stages 36 and 38) were collected from different freshwater pools in Santiago del Estero and Misiones provinces. They were fixed in 10 % formalin.

Sample B. – Nine eggs of *Elachistocleis bicolor* from Corrientes province were reared in the laboratory in a plastic pool with water from the environment where they were collected. Water was changed several times during the next seven days until the larvae reached stage 24. Seven of these tadpoles were placed in a glass container with 250 ml water, and 10 ml of phytoplankton rich in euglenoids (*Euglena ehrenbergii* var. *baculifera* and *Euglena intermedia* var *klebsii*) were added The remaining two control tadpoles were reared separately in another glass container and were fed with commercial fish food and yeast (*Saccharomyces cerevisiae*) The euglenoids' sample and the tadpoles were maintained at 18°C with a photoperiod of 12:12 LD

The intestinal contents of all tadpoles were observed at stages 25, 27 and 36, and from 24 hours to 15 days from the beginning of the experiment. Temporary preparations from the foregut, midgut and hindgut (including the cloaca and vent tube) were made and observed by light microscopy In the temporary preparations, the wall of a small part of the gut was slit and placed on a glass slide, 1-2 drops of water were added, and a cover slip was placed over the material In sample A, intestinal contents and buccopharyngeal cavities were also analyzed by scanning electron microscopy (SEM). Tadpoles preserved in 10 % formalin were entire critical-point dried, and the intestines were removed and broken over a piece of double-sided tape placed on a microscope stub. The intestinal contents were coated with gold-palladium. A Philips 515 scanning electron microscope, a vacuum evaporator (ION Sputtering Balzers SCD 040) and a critical point dryer (Balzers CPD 030) were used.

In sample B, in vivo observations of the intestinal contents were made The algae and protists living in the pond water were identified by light microscopy Two control tadpoles at stage 31, living in the pond, were examined to verify that euglenoids were present in their intestines.

The following bibliography was used for the taxonomic identification of euglenoids GOJDICS (1953), HUBER PESTALOZZI (1955), STARMACH (1983) and TELL & CONFORTI (1986)

The eggs of *E. bicolor* were collected with the permission of and under the rules of the Administración de Parques Nacionales.

Table 1. – Euglenoids found in the gut contents of *Dermatonotus muelleri* and *Elachistocleis bicolor* tadpoles. Parts of intestine: 1, foregut; 2, midgut; 3, hindgut and cloaca.

| Taxon | Part of intestines | | | Observations |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| *Euglena ehrenbergii* var. *baculifera* | | | + | undamaged |
| *Euglena intermedia* var. *klebsii* | + | + | + | alive |
| *Euglena oxyuris* | + | + | + | undamaged |
| *Euglena spiroides* | + | | | undamaged |
| *Lepocinclis fusiformis* | | | + | undamaged |
| *Lepocinclis salina* | | + | + | undamaged |
| *Phacus* sp. | | + | + | undamaged |
| *Phacus curvicauda* | | | + | undamaged |
| *Trachelomonas bacillifera* var. *minima* | + | | | undamaged |
| *Trachelomonas pusilla* | + | | | undamaged |
| *Trachelomonas volvocina* | + | | + | undamaged |

## RESULTS

Intact cells of *Trachelomonas volvocina*, *T. bacillifera* var. *minima* and *T. pusilla* were found in the anterior zone of the intestines of *Dermatonotus muelleri*. *Phacus* sp., *Lepocinclis fusiformis*, *L. salina* and *Trachelomonas volvocina* were found undamaged in the hind gut of fixed tadpoles (sample A) of *D. muelleri* and *Elachistocleis bicolor*. Large numbers of undamaged *Lepocinclis salina*, *L. fusiformis* and *Trachelomonas volvocina* were found in the cloaca. An intact cell was observed inside the lorica of *Trachelomonas volvocina*. *Phacus curvicauda*, *Euglena oxyuris*, *E. ehrenbergii* var. *baculifera*, *Lepocinclis salina* and *L. fusiformis* were found in the posterior part of the intestines of *D. muelleri*. All euglenoids showed an accumulation of paramylon granules. *E. ehrenbergii* var. *baculifera* also contained many carotenoid granules irregularly distributed throughout the cell. SEM observations of the intestinal contents and buccopharyngeal cavities of the tadpoles confirmed the euglenoid taxa identified with light microscopy. *Phacus* sp., *Trachelomonas* sp. and *Euglena spiroides* were observed in the roof of the buccopharyngeal cavity of *Dermatonotus muelleri* (fig. 1a-b). Intact euglenoids were found along with other protists in the anterior and mid-zone of the tadpole intestines. All these cells were undamaged (fig. 1c-d) and they had typical features of free-living cells in nature (fig. 2a-c). Euglenoids were the only cells that were identified from the hindgut (fig. 2d, tab. 1). Living *Euglena intermedia* var. *klebsii* and other algae (species of

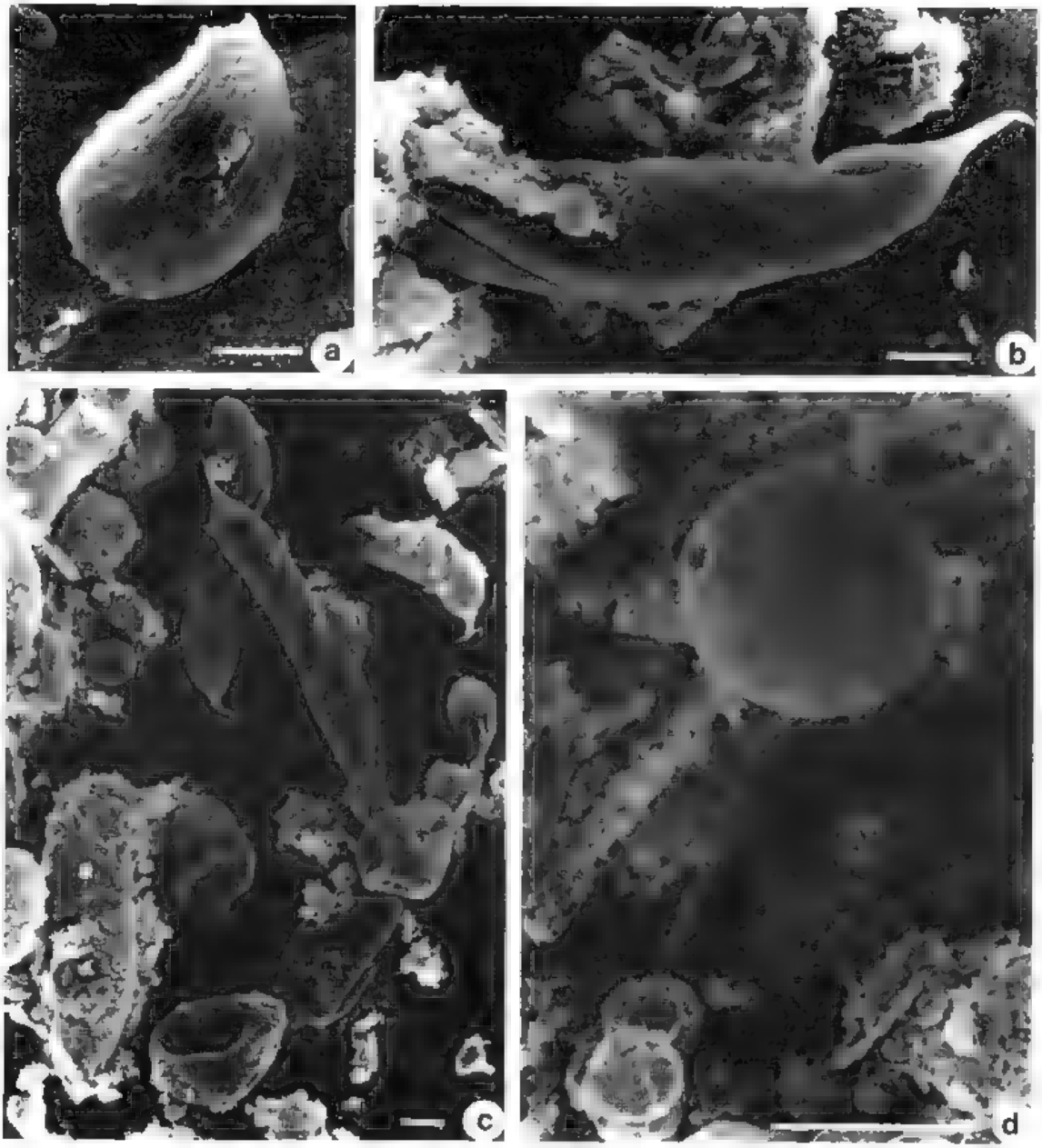


Fig 1 (a) *Phacus* sp. on the wall of the buccopharyngeal cavity of *Dermatomotus muelleri*. Scale line, 10 µm (1550 ×). (b) *Euglena spirodes* in the buccopharyngeal cavity of *D. muelleri*. Scale line, 10 µm (1200 ×). (c) SEM view of several euglenoids in the foregut of *D. muelleri*. Scale line, 10 µm (50 ×). (d) *Trachelomonas pusilla* and several dinoflagellates (*Peridinium* sp.) from the midgut of *D. muelleri*. Scale line, 10 µm (3100 ×).

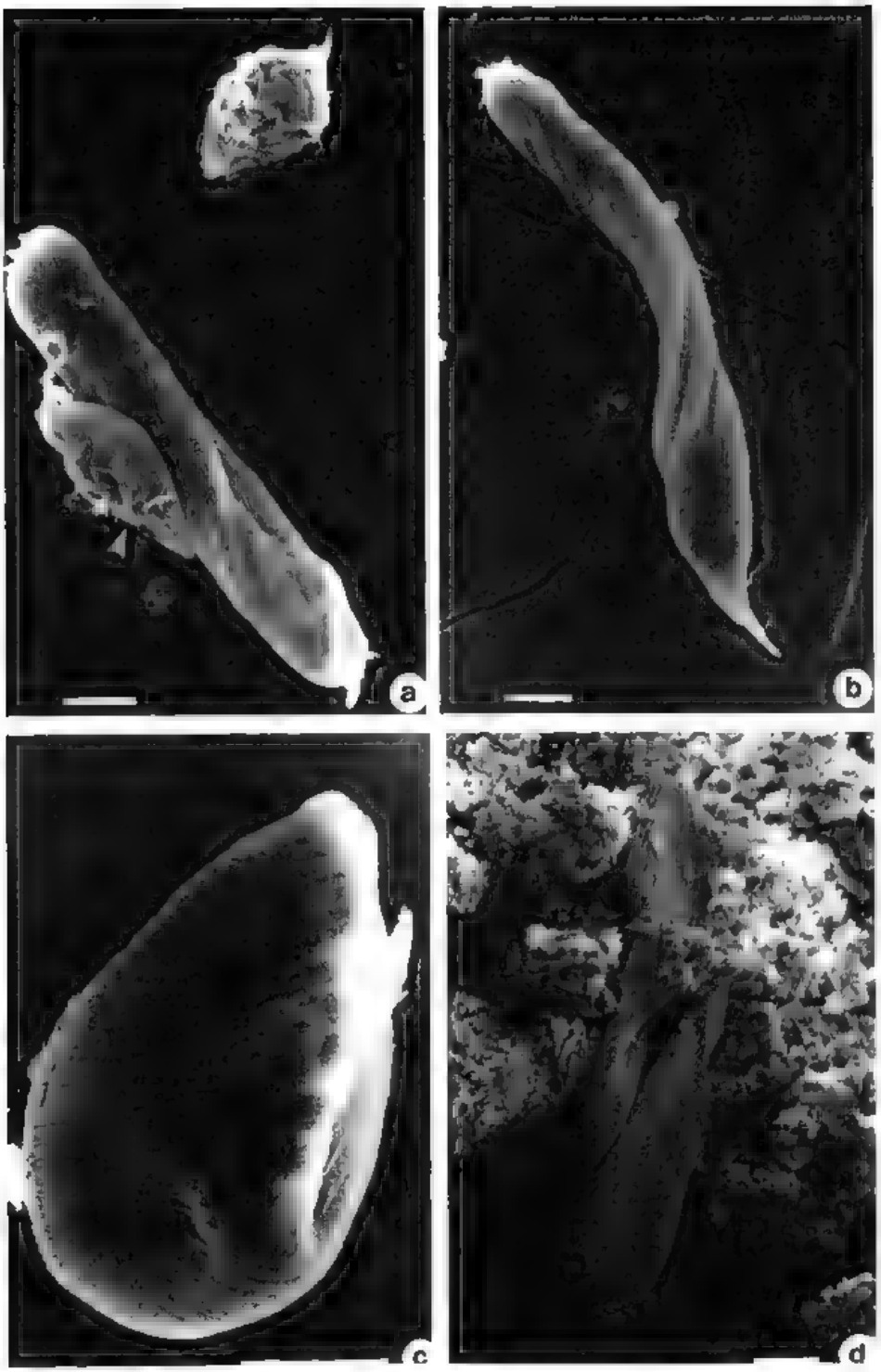


Fig. 2. (a) *Phacus* sp. (arrow) on *Euglena oxyuris* and a contracted *Euglena* sp. from the foregut of *D. muelleri*. Scale line 10 μm (1000 ×). (b) *Euglena spirodes* from the foregut of *D. muelleri*. Scale line 10 μm (1000 ×). (c) *Lepocinclis salina* from the midgut of *Elachistoclers bicolor*. Scale line 10 μm (2400 ×). (d) *Euglena oxyuris* in the hindgut of *D. muelleri*. Scale line, 10 μm. (1000 ×)

Chlorophyceae: *Scenedesmus* sp. and *Ankistrodesmus* sp.; Dinophyceae. *Peridinium* sp.; and Bacillarophyceae: *Nitzschia* sp.) were observed in the foregut and midgut of *Elachistocleis bicolor* (sample B). Only *E. intermedia* var. *klebsii* was found in the hindgut and cloaca. No algae were found in the cloaca other than the living euglenoids. These results were obtained 48 hours, 72 hours and 12 days after the freshwater sample was added to the containers with the tadpoles. The euglenoids were very active, moving inside the intestine, especially in the mid and hindgut, and they occurred in groups over the intestine walls. The intestinal contents of one tadpole of sample B was analyzed 24 hours after placement in the glass container with the phytoplankton. No evidence of *E. ehrenbergii* var. *baculifera* and *E. intermedia* var. *klebsii* were found.

Intestinal contents of four control tadpoles were observed. Two tadpoles were fixed immediately after collection (pond control) from the pond where the eggs of *Elachistocleis bicolor* were obtained. *Euglena spiroides*, *Phacus* sp. and several species of *Trachelomonas* sp. were observed in their intestinal contents. Two embryos were separated from the nine eggs and kept in the absence of euglenoids (intestinal control). Ten days after the experiment began, no euglenoids were observed in the intestinal contents of the developing tadpoles.

## Discussion

Although we found euglenoids in the intestines of the tadpoles of *Dermatonotus muelleri* and *Elachistocleis bicolor*, these protists were not digested by the tadpoles of *E. bicolor*. They were alive and intact inside the length of the intestines. This conclusion specifically applies to *D. muelleri* because all individual euglenoids examined with SEM were undamaged. These tadpoles could be non-selective of the kind of organisms they ingest or digest, which agrees with Carothers & Jaksic (1984). A few authors considered euglenoids to be part of the diet of microhylid tadpoles, but the ability of a given tadpole to use euglenoids as food may be determined by whether the tadpole has a gut laminarase that can degrade paramylon granules (Bull & Chesters, 1966, fide Walne & Kivic, 1990). Euglenoids in *E. bicolor* had an excess of paramylon granules, which suggests that the protists stored paramylon, and the intestines did not limit nutrient availability. This agrees with Conforti (1998) and her results of the study on euglenoids' development in an environment with organic enrichment. Tadpoles are microphagous feeders and the size of *Euglena* is within the range of ingested food particles, but the pellicle and the lorica could be an impediment to digestion. Tadpoles have a non-acid intestinal pH and a long intestine with weak peristalsis (Thrall, 1972, fide Altig & Johnston, 1989). Several enzymes were detected in the intestines of microhylid larvae (Altig & McDiarman, 1975), but euglenoids seem not to be affected by the internal gut conditions. Euglenoids in the intestines had features typical of cells in samples from the field, including pigmented plastids. These features are probably only possible in lighted conditions. The ventral body wall of these tadpoles is translucent, at least in stages 25 to 31, and few coils of the intestines can be seen through the body wall. We presume that sufficient light to promote growth in the protozoa can pass through the tadpole tissues. The large size of paramylon granules indicates that the level of light and nutrients in the intestines provides favorable conditions for the euglenoids. Large accumulations of carotenoid granules in euglenoids were

reported by BOROWITZKA (1988) as a signal of nitrogen deprivation *Euglena ehrenbergii* var *baculifera* in the intestinal contents of *D. muelleri* had a great number of carotenoid granules in the cytoplasm (this was not in the case in *E. bicolor*).

The number of euglenoids present in the last part of the intestines of *D. muelleri* and *E. bicolor* tadpoles suggests that euglenoids could enter the intestines via tadpole's vent tube. The vent tube (and the cloaca) have no muscles nearby. A fecal strand usually extends outside the body and could attract protists inside the cloaca. *Euglena ehrenbergii* may be able to locate the fecal strands by chemoreception of a particular substance, for example, the nitrogenous wastes of *E. bicolor* metabolism. Once inside the intestines, euglenoids could move along the short and transparent intestines coils. In sample B of *E. bicolor*, *Euglena erhrenbergii* var. *baculifera* was absent and only *Euglena intermedia* var. *klebsii* was present. The absence of *E. erhenbergii* var. *baculifera* could be related to its size and/or its ecomorphological type. *E. erhenbergii* var. *baculifera* (188-198 × 19.8-20 μm) is longer than *E. intermedia* var. *klebsii* (78-90 × 7-15 μm). Smaller euglenoids may be more effectively captured by the branchial structures than larger ones, or *E. erhenbergii* var. *baculifera* may not be harvested by these suspension-feeding tadpoles because of its benthic habitat. The results of our in vivo study provide new information on the diet of two filter feeding tadpoles of Argentina.

## CONCLUSIONS

(1) Euglenoids, along with other algae (several species of Chlorophyceae, Dinophyceae and Bacillariophyceae) were found alive and undamaged in the intestinal contents examined by optical microscopy.

(2) At least the observed euglenoids are not digested because they were always found intact and alive inside the gut (anterior, posterior and cloacal portions). Euglenoid flagellates were the only protists found living in the cloaca.

(3) The storage of paramylon granules in euglenoids suggests that the intestinal conditions were favorable for these organisms, and that they encountered no nutrient limitation.

(4) The presence of live, undamaged euglenoids indicates that they are not part of the diet of these tadpoles.

## RÉSUMÉ

Dans le contenu intestinal de têtards de *Dermatonotus muelleri* et de *Elachistocleis bicolor* provenant de divers environnements aquatiques, nous avons trouvé une grande quantité d'euglénoïdes flagellés (*Euglena ehrenbergii* var. *baculifera*, *E. spiroides*, *Lepocinclis fusiformis*, *L. salina*, *Phacus curvicauda*, *Trachelomonas bacillifera* var. *minima*, *T. pusilla* et *T. volvocina*). Au laboratoire, 9 oeufs de *Elachistocleis bicolor*, récoltés dans des environnements naturels, se sont développés jusqu'au stade 24 (selon GOSNER, 1980). Les têtards ont été alimentés avec du phytoplancton très riche en euglenoïdes flagellés qui contenait *Euglena intermedia* var. *klebsii*

et *Euglena ehrenbergii* var *baculifera*. Après 48 h, 72 h et 12 jours, nous avons enregistré la présence de *E. intermedia* var *klebsii* vivante à l'intérieur de l'intestin Etant donné que les plastides étaient intacts et que les corps de paramylon étaient similaires à ceux qu'on trouve dans la nature, les algues ne semblent pas affectées par le milieu intérieur de l'intestin. Ces résultats nous permettent de conclure que les euglénoïdes étudiés ne font pas partie du régime alimentaire des larves de ces Microhylidés et supportent sans problème apparent les conditions internes de la cavité intestinale.

## Resumen

En el contenido intestinal de renacuajos de *Dermatonotus muelleri* y de *Elachistocleis bicolor* hallamos gran acumulación de euglenoideos flagelados (*Euglena ehrembergii* var *baculifera*, *E. oxyuris*, *E spiroides*, *Lepocinclis fusiformis*, *L. salina*, *Phacus curvicauda*, *Trachelomonas baculifera* var. *minima*, *T pusilla* and *T. volvocina*), provenientes de distintos cuerpos de agua. En el laboratorio, se dejaron desarrollar 9 huevos de *Elachistocleis bicolor*, recogidos en ambientes naturales, hasta el estadio 24 (según Gosner, 1980) Los renacuajos fueron alimentados con fitoplancton muy rico en euglenoideos flagelados que contenía principalmente *Euglena intermedia* var. *klebsii* y *E ehrembergii* var, *baculifera*. A las 48 horas, 72 horas y 12 días se registró la presencia de *E intermedia* var. *klebsii*, vivas en el interior del intestino. Ellas no mostraron signos de ser afectadas por el medio interno del intestino ya que presentaron los plástidos intactos y cuerpos de paramilon similares a los hallados en la naturaleza Nuestros resultados permiten concluir que los euglenoideos estudiados no formarían parte de la dieta de los renacuajos de microhylidos mencionados, y que soportan sin perjuicio aparente las condiciones internas de la cavidad intestinal.

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## Literature cited

Altig, R & Johnston, G F 1989 Guilds of anuran larvae relationships among developmental modes, morphologies, and habitats. *Herp. Mon.*, **3**. 81-109

Altig, R & Mc Diarman, W 1975 Clearance times and percent assimilation of five species of anuran tadpoles. *Herpetologica*, **31** 67-69

Borowitzka, M A., 1988. – Vitamins and fine chemicals from microalgae *In* M Borowitzka & L. Borrow (ed.), *Micro-algal biochemistry*, Cambridge Univ. Press 152-196

BRUMPT, E & LAVIER, G , 1924. Un nouvel Euglenien polyflagellé parasite du têtard de *Leptodactylus ocellatus* au Brésil *Annales de Parasitologie Humaine et Comparée*, **2**: 248-252.

BULL, J. T. & CHESTERS, C G C , 1966 The biochemistry of laminarin and nature of laminarase. *In*. S. S. NORD (ed), *Advances in enzymology and related subjects in biochemistry*, New York, Wiley: 325-364

CAROTHERS, J. H. & JAKSIC, F M , 1984. Time as a niche difference the role of interference competition *Oikos*, **42**: 403-406.

CONFORTI, V., 1998. Morphological changes of Euglenophyta in response to organic enrichment *Hydrobiologia*, **369-370**: 277-285.

GOJDICS, M , 1953. *The genus* Euglena Madison, The University of Wisconsin Press 1-268

GOSNER, K. L., 1960 A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**: 183-190.

HEGNER, R W, 1926 – The interrelations of protozoa and utricles of *Utricularia Biol Bull.*, **50**: 239-270

HEYER, W. R , 1973 – Ecological interactions of frog larvae at a seasonal tropical location in Thailand *J. Herp.*, **7**: 337-361.

HUBER-PESTALOZZI , G , 1955. – Die Binnengewässer. *Das Phytoplankton des Susswassers*, Stuttgart, **16** (4): 1-1135

INGER, R F , 1986 Diets of tadpoles living in a Bornean rain forest *Alytes*, **5** (4). 153-164.

INGER, R F , VORIS, W K & FROGNER, K, F , 1986 Organization of a community of tadpoles in rain forest streams in Borneo. *J. tropical Ecol* , **2**: 193-205

LI, J & C S. LIN, 1935. Studies on the rain frog, *Kaloula borealis* II The food and feeding of the embryos and adults. *Peking nat. Hist. Bull* , **10**: 45-53

SAVAGE, R M , 1952. - Ecological, physiological and anatomical observations on some species of anuran tadpoles. *Proc. zool. Soc. London*, **122**. 467-514

STARMACH, K , 1983 - Euglenophyta Euglininy *Flora slodkowodna Polski*, P W N Warszawa, **3**. 1-594

TELL, G & CONFORTI, V., 1986. *Euglenophyta pigmentadas de la Argentina* Berlin, Bibliotecа Phycologica, **75**: 1-301.

THRALL, J. H , 1972 *Food feeding and digestive physiology of the larval bullfrog*, Rana catesbeiana *Shaw* PhD Dissertation, Illinois State University, Bloomington.

WALNE, P L & KIVIC P A , 1990 - Phylum Euglenida *In* JONES & BARTLETT (ed ), *Handbook of Protoctista*, Boston, **15**. 270-287.

WANG, C S., WU, S. H & YU, W T, 1989 Notes on *Microhyla inornata* Boulenger (Anura, Microhylidae) in Taiwan. *J. Herp.*, **23** (4): 342-349

WENRICH, D H , 1924 – Studies on *Euglenamorpha hegneri* n g , n sp., a euglenoid flagellate found in tadpoles. *Biol. Bull* , **47**: 149-174

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